Research Article

Received: 20 January 2010 Revised: 28 February 2010 Accepted: 16 March 2010 Published online in Wiley Interscience: 26 April 2010

(www.interscience.wiley.com) DOI 10.1002/jctb.2412

Kinetic study of nordihydroguaiaretic acid recovery from *Larrea tridentata* by microwave-assisted extraction

Silvia Martins,[a] Cristóbal N. Aguilar,[b] Iliana de la Garza-Rodriguez,[c] Solange I. Mussatto[a]* and José A. Teixeira[a]

Abstract

BACKGROUND: Nordihydroguaiaretic acid (NDGA) is a powerful antioxidant with biological activities of great interest in several health areas, including antiviral, cancer chemopreventive, and antitumorgenic. Little information is available on extraction methods of NDGA from *Larrea tridentata*. Hence, the aim of this study was to develop a rapid and effective microwave-assisted extraction (MAE) method for NDGA recovery from *Larrea tridentata* leaves, and to compare the results obtained with those found using conventional heat-reflux extraction (HRE).

RESULTS: Extraction time for similar NDGA yields was significantly reduced from 18 to 1 min when MAE was used instead of HRE. Optimum conditions for NDGA extraction (3.79 ± 0.65%) consisted in using 50% methanol as extraction solvent in a solid/liquid ratio of 1/10 g mL^{-1}. Micrographs demonstrated that the improvement in NDGA extraction by MAE might be related to a greater extent of cell rupture of the plant material. Extracts obtained by MAE exhibited antiradical activity only slightly lower than those obtained by HRE.

CONCLUSIONS: MAE proved to be a faster and more efficient method for NDGA extraction from *Larrea tridentata* leaves than HRE. The better results for NDGA extraction by MAE might be explained by the greater extent of cell rupture of plant material during the extraction process.

Keywords: nordihydroguaiaretic acid; *Larrea tridentata*; microwave-assisted extraction; heat-reflux extraction

INTRODUCTION

Nordihydroguaiaretic acid (NDGA) is a lignan found in several plants, including *Larrea tridentata* (*Zygophyllaceae*), also known as creosote bush, which grows in semidesert areas of south-western USA and Northern Mexico.[1] NDGA can be found in flowers, leaves, green stems and small woody stems. In *Larrea tridentata* it is mainly concentrated in the leaves (38.3 mg g^{-1}) and green stems (32.5 mg g^{-1}).[2] The higher concentrations of these compounds in leaves and green stems is because lignans are considered natural defense substances of photosynthetic tissue in plants, which are more exposed to UV radiation, climatic changes, herbivores and pathogens attacks.[2–4] In addition, the concentration of secondary metabolites (like NDGA) in plants might be influenced by several other factors, namely, physiological variations, environmental conditions, geographic variations, genetic factors, and evolution.[5] NDGA is known to be a powerful antioxidant;[6] however, recent studies have shown other very important biological activities for this compound, such as antiviral, cancer chemopreventive, and antitumorgenic activities.[7–9]

Extraction of bioactive compounds from plants is conventionally performed by heat-reflux systems, which usually are time consuming and require large amounts of solvent. Several parameters, including extraction temperature, solvent concentration and solid/liquid ratio play an important role in the extraction of bioactive compounds.[10] The increased need for an ideal extraction method that allows maximum bioactive compound recovery from a plant in the shortest processing time with low costs, represents an important challenge. Different techniques for bioactive compounds extraction have been proposed, including ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, and high pressure processing.[11–14] Among these, microwave-assisted extraction (MAE) has significantly decreased extraction time and increased extraction yields in several plants.[15–18] When MAE is applied, the solvent choice

* *Correspondence to: Solange I. Mussatto, Institute for Biotechnology and Bioengineering (IBB), Centre of Biological Engineering, University of Minho, Campus Gualtar, 4710-057, Braga, Portugal.*
E-mail: solange@deb.uminho.pt; solangemussatto@hotmail.com

a *Institute for Biotechnology and Bioengineering (IBB), Centre of Biological Engineering, University of Minho, Campus Gualtar, 4710-057, Braga, Portugal*

b *Food Research Department, School of Chemistry, Autonomous University of Coahuila, Blvd. Venustiano Carranza S/N Col. República Oriente, 25280, Saltillo, Coahuila, Mexico*

c *Laboratory of Environmental Analytical Chemistry, School of Chemistry, Autonomous University of Coahuila, Blvd. Venustiano Carranza S/N Col. República Oriente, 25280, Saltillo, Coahuila, Mexico*

is determined by the solubility of the extracts of interest, the interaction between solvent and plant matrix, and the microwave absorbing properties of the solvent, determined by its dielectric constant.[19] NDGA is characterized for its insolubility in water and solubility in organic solvents such as ethanol and methanol (supplier specifications). It is well known that extracting solvents with high dielectric constant have a greater ability to absorb microwave energy;[20] and high microwave energy absorption results in fast dissipation of energy into the solvent and solid plant matrix, which generates an efficient and homogeneous heating.[21]

There is little information available on NDGA extraction from *Larrea tridentata*. To our knowledge, no studies of the MAE method for NDGA recovery from *Larrea tridentata* leaves have been reported. Thus, the aim of this work was to develop a MAE technique for efficient NDGA extraction from *Larrea tridentata* leaves and to compare the results obtained with those found using conventional heat-reflux extraction.

EXPERIMENTAL

Plant material and chemicals

Plant material (*Larrea tridentata*) was collected from the Chihuahuan semidesert (North Coahuila, Mexico) during Spring season (April, 2008).

Nordihydroguaiaretic acid (high purity) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). HPLC-grade methanol and acetonitrile were purchased from Fermont (Monterrey, NL, Mexico). Reagent-grade methanol was purchased from Jalmek (Monterrey, NL, Mexico) and acetic acid from CTR (Monterrey, NL, Mexico).

Extraction methodologies

Air-dried leaves of *Larrea tridentata* were ground to fine powder and stored in dark bottles at room temperature for further analysis. Since methanol has a higher dielectric constant than ethanol (32.6 and 24.3, respectively), it was chosen as the extraction solvent for NDGA recovery. In the present study, an extraction temperature of 70 °C was selected considering the boiling point of the extraction solvent (methanol, 64.7 °C), and because no problems with pressure in the extraction vessel occurred, which could damage the microwave equipment.[22]

Conventional heat-reflux extraction was performed mixing 1 g of dried powdered plant with the solvent (solid/liquid ratio 1/10 g mL^{-1}), in 250 mL Erlenmeyer flasks, which were covered with foil paper to prevent light exposure and subsequent oxidation.[23] Reactions were performed in a water-bath at 70 ± 2 °C, using different methanol concentrations as solvent (25 to 100% v/v) for 1 or 3 h. During conventional extraction by reflux, the temperature was monitored using a thermocouple data logger (USB TC-08, Pico Technology, UK), which was placed inside the flask containing the sample. After 14 min, approximately, the desired temperature was achieved and the extraction time started. Data were registered by a PC and a temperature profile was obtained. The heat transfer coefficient k was calculated, obtaining a value of $4.17 \times 10^{-3} \pm 1.4 \times 10^{-4}$ s^{-1}.

Microwave-assisted extraction was carried out in a microwave apparatus using a multimode closed vessel system with pressure (Microwave Digestion Unit, CEM MARS Express, USA). For reactions, 1 g of dried powdered plant was mixed with the desired amount of solvent and placed into 100 mL polytetrafluoroethylene (PTFE) extraction vessels. The suspensions were irradiated with microwaves at 70 ± 2 °C and 800 W, according to the method of Zhang *et al*.[21] with some modifications. After each irradiation of 1 min at a power of 800 W the sample was allowed to cool to room temperature. Different methanol concentrations as solvent (25 to 100% v/v) and solid/liquid ratios (1/5 to 1/30 g mL^{-1}) were tested.

The extracts obtained by both methods were filtered using a muslin cloth and filter paper to remove macro-particles. Before high performance liquid chromatography (HPLC) analysis all the extracts were filtered through a 0.2 µm membrane filter. A total of three extracts were prepared and all analyses were performed in triplicate. NDGA yield (w/w) was defined as the ratio between mass of NDGA in the extracts and mass of plant material, ×100%.

HPLC analysis

NDGA concentration in the obtained extracts was determined by HPLC using a Varian ProStar 3300 system (Chicago, IL, USA), equipped with a pump (ProStar 230 SDM), an auto sampler (ProStar 410 AutoSampler), and a UV-photodiode array detector (PDA ProStar 350) at 280 nm. Data acquisition was made using the LC Workstation software (Version 6.2). Chromatographic separation was carried out in an Optisil ODS reversed-phase column (5 µm; 250 × 4.6 mm) at 31 °C, using a mobile phase consisting of acetonitrile (solvent A) and 0.3% acetic acid in water (v/v) (solvent B) under the following gradient profile: 30% A/70% B (0–2 min), 50% A/50% B (2–11 min), 70% A/30% B (11–17 min), 100% A (17–22 min), and 30% A/70% B (22–40 min). The mobile phase was eluted in a flow rate of 1.0 mL min^{-1}, and samples of 10 µL were injected.[24]

Determination of kinetic parameters and extraction time

The experimental data were fitted to a first-order kinetic model to describe the NDGA extraction process:

$$\mathrm{NDGA} = \mathrm{NDGA}_{\infty} \times \left(1 - e^{-\mathrm{k.t}}\right) \quad (1)$$

where $NDGA_{\infty}$ is the NDGA recovered after the extraction process, k (min^{-1}) is the first-order extraction rate constant, and t (min) the time. By rearranging Equation (1), it is possible to determine the time at which the extraction process reaches equilibrium using the following equation:

$$\mathrm{t} = -\frac{1}{k} \times \ln\left(1 - \frac{\mathrm{NDGA}}{\mathrm{NDGA}_{\infty}}\right) \quad (2)$$

Since $\frac{\mathrm{NDGA}}{\mathrm{NDGA}_{\infty}} \cong 0.99$, the extraction time for both HRE and MAE methods is easily determined.

Scanning electron microscopy

Micrographs of plant material samples (untreated and treated by MAE and HRE) were obtained by scanning electron microscopy using a Leica Cambridge S360 microscope. The samples were prepared as described by Zhang *et al*.[21] with some modifications. Briefly, after solvent removal, the plant material was plunged in liquid nitrogen and then cut with a scalpel. The sectioned pieces were fixed on a specimen holder with aluminum tape and then sputtered with platinum in a sputter-coater under high vacuum condition. All the specimens were examined at 500× magnification.

Free radical scavenging effectiveness of *Larrea tridentata* extracts

The free radical effectiveness of *Larrea tridentata* extracts obtained by MAE and HRE was determined and compared by measuring the ability of the extracts to scavenge the free radical DPPH (1,1-diphenyl-2-picrylhydrazyl). The DPPH radical scavenging activity was determined as described by Szabo *et al.*[25] with modifications. 100 µL of each extract, duly diluted in methanol at concentrations ranging from 5 to 100 mg L^{-1}, was added to 2.9 mL of DPPH solution (6×10^{-5} mol L^{-1} in methanol). The resulting solutions were vortexed, and allowed to stand for 30 min in darkness at room temperature. The absorbance was measured at 517 nm in a spectrophotometer (Biomate 3, UV-visible spectrophotometer, NY, USA), using methanol as blank. The control solution consisted in using methanol instead of the sample. All analyses were performed in quadruplicate.

The radical scavenging activity was expressed as the inhibition percentage using the following equation:

$$\%\ DPPH\ radical\ scavenging = (1 - A_S/A_C) \times 100 \qquad (3)$$

where A_C and A_S are the absorbance of the control solution and the absorbance of the sample solutions, respectively. The effectiveness of the extracts of *Larrea tridentata* leaves obtained by MAE and HRE in scavenging free radicals was evaluated as the concentration (mg L^{-1}) of extract in the reaction mixture required to scavenge 50% of DPPH free radical, defined as EC_{50} ('effectiveness concentration' value). This parameter (EC_{50}) was calculated from the inhibition curve plotting the DPPH radical scavenging percentage against the extracts concentration. NDGA was used as positive control.

Statistical analysis

Results were analyzed by one-way analysis of variance (ANOVA) in the general linear model of SPSS (Statistical Package for Social Sciences, version 16.0), employing a significance level of $P < 0.05$. The difference among samples was verified using Tukey's range test.

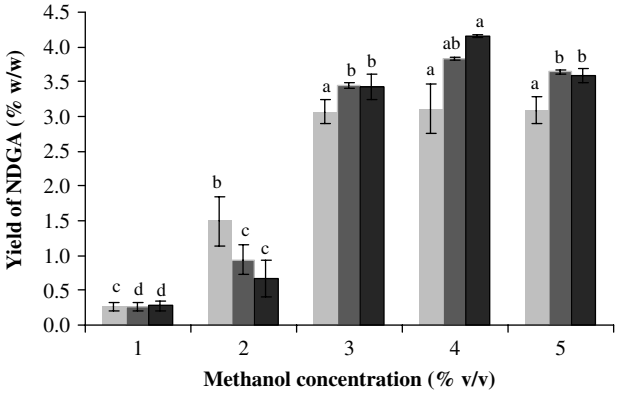

Figure 1. Effect of methanol concentration on NDGA extraction from *Larrea tridentata* leaves by MAE (light shading) (1 g plant 30 mL^{-1} solvent, 70 °C, 800 W, for 4 min); and HRE (medium shading) 1 h and (darkest shading) 3 h (1 g plant 30 mL^{-1} solvent, 70 °C). [abcd] Values in a column with the same superscripts are not significantly different at $P < 0.05$.

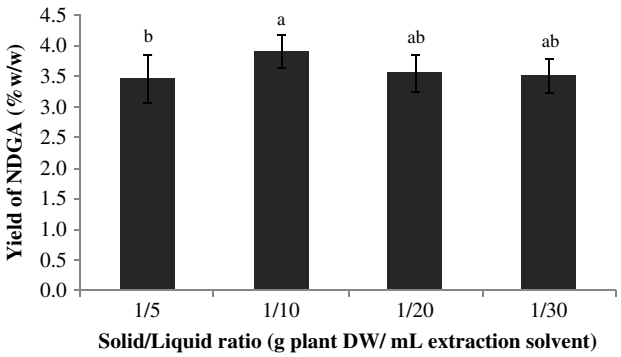

Figure 2. Effect of solid/liquid ratio on NDGA extraction from *Larrea tridentata* leaves by MAE using methanol 50% (v/v) as solvent, at 70 °C, 800 W, for 4 min. [ab] Values in a column with the same superscripts are not significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

Parameters affecting the NDGA extraction

Methanol concentration had a strong influence on NDGA extraction from *Larrea tridentata* leaves using MAE and HRE (Fig. 1). During MAE the NDGA yield significantly increased ($P < 0.05$) when a methanol concentration of 50% was used instead of water or a methanol concentration of 25%. However, there was no significant difference ($P < 0.05$) in NDGA yields when methanol concentrations higher than 50% were used. These findings showed that the addition of some water resulted in enhancement of the extraction efficiency, possibly due to the increase in plant material swelling in the presence of water, increasing the contact surface area between the plant matrix and the solvent.[26,27] In the case of HRE, the process was performed for 1 or 3 h to determine if a larger extraction time could have any effect on NDGA recovery, but no significant differences ($P < 0.05$) in NDGA yields were observed. Notwithstanding, NDGA yield was affected by the methanol concentration, the values being significantly higher when a methanol concentration of 50% was used for 1 h, compared with water or a methanol concentration of 25%. There was no significant difference in NDGA yields when methanol concentrations of 75 or 100% were used compared with a methanol concentration of 50%. In brief, methanol in water at 50% v/v was the best extraction solvent for both, HRE and MAE techniques.

Some studies report that the solid/liquid ratio affects the bioactive compounds yield during MAE.[15,21] The effect of solid/liquid ratio on the NDGA yields during MAE from *Larrea tridentata* leaves is shown in Fig. 2. In fact, it can be observed that using a solid/liquid ratio of 1/10 (g mL^{-1}) resulted in significantly higher ($P < 0.05$) NDGA yields compared to a solid/liquid ratio of 1/5. However, there was no significant difference in NDGA yields using solid/liquid ratios of 1/20 or 1/30. Therefore, 1/10 (g dried plant material mL^{-1} extraction solvent) was considered the best solid/liquid ratio to be used during MAE of NDGA from *Larrea tridentata* leaves for 4 min at 70 °C. From an economic point of view the use of high solid/liquid ratios in industrial processes is not favored because it can lead to downstream processing problems. In order to overcome this problem, the application of lower solid/liquid ratios might represent an alternative for satisfactory NDGA recoveries for industrial applications, since the results in the present study showed no significant differences ($P < 0.05$) between results when solid/liquid ratios of 1/10, 1/20 and 1/30 were used (Fig. 2).

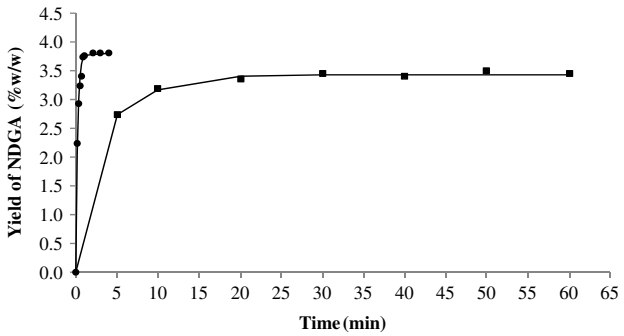

Figure 3. Kinetic behavior of NDGA extraction from *Larrea tridentata* leaves by MAE (●) and HRE (■) using 1 g plant material 10 mL^{-1} methanol 50% (v/v), at 70 °C and 800 W. The symbols represent the experimental NDGA values and the solid line represents the fitted data to a first-order kinetic model (Equation (2)).

Table 1. Kinetic parameters and extraction times obtained for NDGA extracted from *Larrea tridentata* leaves by HRE and MAE

Extraction method	$NDGA_\infty$ (%, w/w)[a]	K (min^{-1})	R^2	Extraction time (min)
HRE	3.42 ± 0.19	0.26 ± 0.02	0.9987	18
MAE	3.79 ± 0.65	4.61 ± 0.45	0.9948	1

[a] NDGA recovered after the extraction process.
K is first-order extraction rate constant.
R is correlation factor for the adjustment of experimental NDGA values to the first-order kinetic model.

Comparison of NDGA extraction by MAE and HRE

Figure 3 shows the kinetic behavior of NDGA extraction from *Larrea tridentata* leaves by MAE and HRE carried out for 4 and 60 min, respectively. Kinetic parameters and extraction time for both MAE and HRE methods are presented in Table 1. Note that MAE was more advantageous than HRE since it reduced the extraction time from 18 to 1 min only, presenting, as a consequence, a higher extraction rate constant (4.61 ± 0.45 min^{-1}). Moreover, slightly higher NDGA yields were found when using MAE as an extracting technique. These results are consistent with those reported by other authors using different plant materials. For example, Zhang *et al.*[21] showed that MAE significantly reduced the extraction time of chlorogenic acid from flower buds of *Lonicera japonica* to 5 min in comparison with 30 min by conventional HRE, and gave higher extraction efficiency. Zhou and Liu[28] reported MAE to be a faster extraction technique for solanesol extraction from tobacco leaves than conventional heat-reflux, since it reduced to 40 min the 180 min required by conventional HRE. MAE was also a faster and more efficient technique for the extraction of flavonoids from *Radix Astragali* compared with conventional HRE, reducing the extraction time from two 2 h cycles to two 25 min cycles, and increasing the percentage flavonoids extraction.[29]

A possible explanation for the better results for MAE compared with HRE, could be an efficient dissipation and absorption of microwave energy through the solvent and plant material, which increases the temperature inside the plant cells. This might result in cell walls breaking, allowing the release of bioactive compounds into the surrounding solvent. Therefore, in order to understand the mechanisms of MAE and HRE, samples of plant material

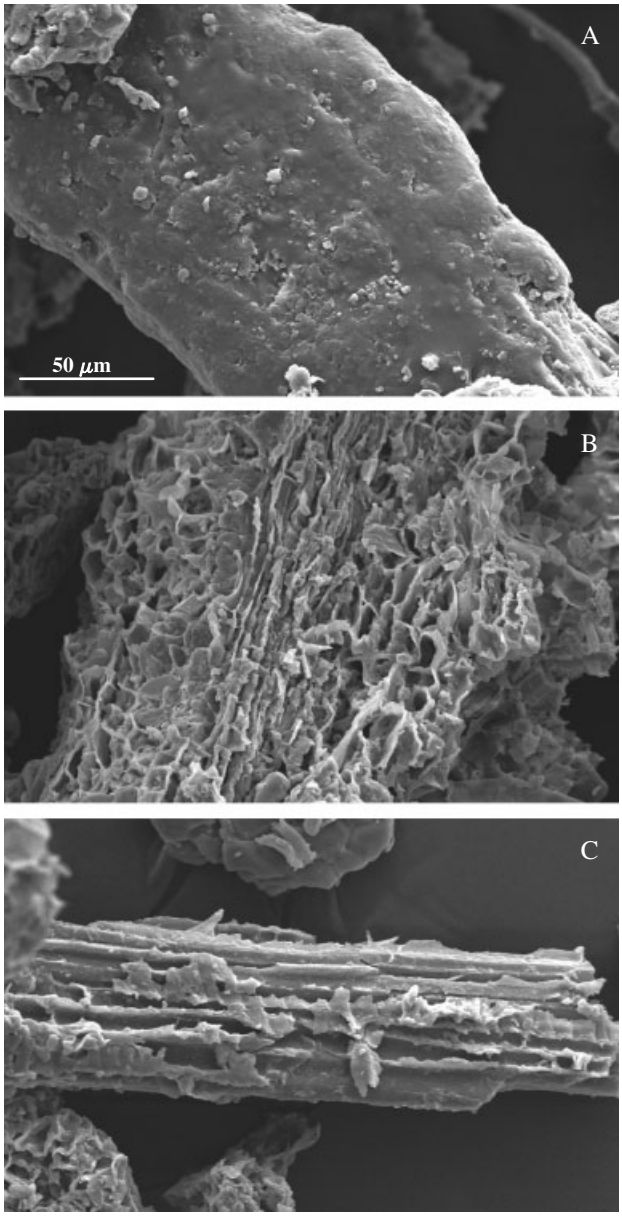

Figure 4. SEM micrographs, of *Larrea tridentata* samples in the following forms: (A) untreated; (B) after MAE; and (C) after conventional HRE. Magnification: 500×.

treated by these two techniques were examined by scanning electron microscopy, and compared with an untreated plant material sample. Analysis of these micrographs clearly revealed much greater destruction of the material surface treated by MAE (Fig. 4(C)) than by HRE (Fig. 4(B)). In the original form (Fig. 4(A)) the material was a rigid structure, which was affected by the HRE treatment. However, MAE treatment was able to destroy the plant structure, probably due to the sudden temperature rise and the internal pressure increase. Similar results were also found in other studies with MAE of solanesol from tobacco leaves,[28] scutellarin from *Erigeron breviscapus*,[30] and chlorogenic acid from flower buds of *Lonicera japonica*.[21] It was thus concluded that the improvement in NDGA extraction by MAE when compared with HRE is related to the greater extent of cell rupture of the plant material.

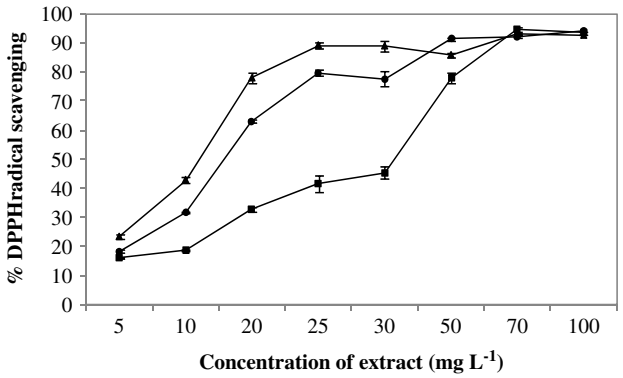

Figure 5. Effect of different concentrations of extracts obtained by MAE and HRE from *Larrea tridentata* leaves in free radical DPPH scavenging activity (■ NDGA positive control, ▲ extract obtained by HRE, ● extract obtained by MAE).

Effectiveness of *Larrea tridentata* extracts on free radical scavenging

DPPH assay is a test able to evaluate the antioxidant potential of extracts,[25] and was thus used in the present work for evaluation of the effectiveness of extracts from *Larrea tridentata* leaves obtained by MAE and HRE, on free radical scavenging (Fig. 5). The lower the EC_{50} (extract in the reaction mixture required to scavenge 50% of DPPH free radical) value the higher the free radical scavenging activity of an extract. According to the results, the EC_{50} for the extract obtained by MAE was slightly higher than that for the extract obtained by HRE (15.57 ± 0.16 and 12.52 ± 0.31 mg L^{-1}, respectively), and consequently, the antiradical activity was slightly lower (Fig. 5). Such results could be due to the microwave irradiation, which might degrade the antiradical activity of the respective extracts. These findings are consistent with those of Hemwimon *et al*.[20] who reported that extracts of anthraquinones obtained by MAE from roots of *Morinda citrifolia* had slightly higher EC_{50} values than those obtained by conventional soxhlet extraction method.

While extracts obtained by MAE presented the highest DPPH radical scavenging activity of 91.44% at 50 mg L^{-1}, extracts obtained with HRE had a lower antiradical activity of 85.76% for the same concentration (Fig. 5). When a 70 mg L^{-1} concentration was applied, similar DPPH radical scavenging activity of the extracts obtained by MAE was observed compared with that achieved at 50 mg L^{-1} (92.25%); nevertheless, extracts obtained by HRE exhibited higher DPPH radical scavenging activity (92.94%). Additionally, the EC_{50} for the solution of NDGA used as positive control was higher than those obtained for the extracts obtained by MAE and HRE (32.53 ± 0.39 mg L^{-1}), which means that the positive control of NDGA had a lower DPPH radical scavenging activity. Such findings were expected since extracts obtained by MAE and HRE from *Larrea tridentata* leaves are composed of several polyphenolic compounds other than NDGA that possess antiradical activity.

In brief, microwave-assisted extraction was a faster and more efficient method for NDGA extraction from *Larrea tridentata* leaves than conventional heat-reflux extraction, mainly because it significantly reduced the extraction time. Under the optimal MAE conditions (50% methanol in water (v/v) as extraction solvent, solid/liquid ratio of 1/10 (g mL^{-1}), 70 °C, for 1 min), maximum NDGA yield of 3.79 ± 0.65% was achieved. The better results of NDGA extraction by MAE might be related to a greater extent of cell rupture of the plant material, which was observed by scanning electron microscopy. Finally, extracts obtained from *Larrea tridentata* leaves using the MAE technique appear to have antioxidant potential, since they presented antiradical activity. However, further studies are needed in order to support this idea and to evaluate the ability of purified fractions of NDGA from MAE extracts to scavenge other free radicals.

ACKNOWLEDGEMENTS

The authors are grateful to FCT (Contract/grant number: SFRH/BD/40439/2007) for financial support of this work.

REFERENCES

1 Ross IA, *Medicinal Plants of the World – Chemical Constituents, Traditional and Modern Medicinal Uses*, Vol 3. Humana Press, New Jersey (2005).
2 Hyder PW, Fredrickson EL, Estell RE, Tellez M and Gibbens RP, Distribution and concentration of total phenolics, condensed tannins, and nordihydroguaiaretic acid (NDGA) in creosotebush (*Larrea tridentata*). *Biochem Syst Ecol* **30**:905–912 (2002).
3 Buranov AU and Mazza G, Lignin in straw of herbaceous crops. *Ind Crops Prod* **28**:237–259 (2008).
4 Bennett N and Wallsgrove RM, Secondary metabolism in plant defence mechanisms. *New Phytol* **127**:617–633 (1994).
5 Figueiredo AC, Barroso JG, Pedro LG and Scheffer JJC, Factors affecting secondary metabolite production in plants: volatile components and essential oils. *Flavour Frag J* **23**:213–226 (2008).
6 Moody TW, Leyton J, Martinez A, Hong S, Malkinson A and Mulshine JL, Lipoxygenase inhibitors prevent lung carcinogenesis and inhibit non-small cell lung cancer growth. *Exp Lung Res* **24**:617–628 (1998).
7 Hwu JR, Hsu MH and Huang RC, New nordihydroguaiaretic acid derivates as anti-HIV agents. *Bioorg Med Chem Lett* **18**:1884–1888 (2008).
8 Cui Y, Lu C, Liu L, Sun D, Yao N, Tan S, *et al*, Reactivation of methylation-silenced tumor suppressor gene p161NK4a by nordihydroguaiaretic acid and its implication in G1 cell cycle arrest. *Life Sci* **82**:247–255 (2008).
9 Toyoda T, Tsukamoto T, Mizoshita T, Nishibe S, Deyama T, Takenaka Y, *et al*, Inhibitory effect of nordihydroguaiaretic acid, a plant lignan, on *Helicobacter pylori*-associated gastric carcinogenesis in Mongolian gerbils. *Cancer Sci* **98**:1689–1695 (2007).
10 Wang L and Weller CL, Recent advances in extraction of nutraceuticals from plants. *Trends Food Sci Technol* **17**:300–312 (2006).
11 Jun X, Caffeine extraction from green tea leaves assisted by high pressure processing. *J Food Eng* **94**:105–109 (2009).
12 Ma YQ, Chen JC, Liu DH and Ye WQ, Simultaneous extraction of phenolic compounds of citrus peel extracts: effect of ultrasound. *Ultrason Sonochem* **16**:57–62 (2009).
13 Lianfu Z and Zelong L, Optimization and comparison of ultrasound/microwave assisted extraction (UMAE) and ultrasonic assisted extraction (UAE) of lycopene from tomatoes. *Ultrason Sonochem* **15**:731–737 (2008).
14 Pascual-Martí MC, Salvador A, Chafer A and Berna A, Supercritical fluid extraction of resveratrol from grape skin of *Vitis vinifera* and determination by HPLC *Talanta* **54**:735–740 (2001).
15 Proestos C and Komaitis M, Application of microwave-assisted extraction to the fast extraction of plant phenolic compounds. *Food Sci Technol (LWT)* **41**:652–659 (2008).
16 Rostagno MA, Palma M and Barroso CG, Microwave assisted extraction of soy isoflavones. *Anal Chim Acta* **588**:274–282 (2007).
17 Pan X, Niu G and Liu H, Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chem Eng Process* **42**:129–133 (2003).
18 Guo Z, Jin Q, Fan G, Duan Y, Qin C and Wen M, Microwave-assisted extraction of effective constituents from a Chinese herbal medicine Radix puerariae. *Anal Chim Acta* **436**:41–47 (2001).
19 Brachet A, Christen P and Veuthey J-L, Focused microwave-assisted extraction of cocaine and benzoylecgonine from coca leaves *Phytochem Anal* **13**:162–169 (2002).

20 Hemwimon S, Pavasant P and Shotipruk A, Microwave-assisted extraction of antioxidantive anthraquinones from roots of *Morinda citrifolia*. *Sep Purif Technol* **54**:44–50 (2007).
21 Zhang B, Yang R and Liu C-Z, Microwave-assisted extraction of chlorogenic acid from flower buds of *Lonicera japonica Thunb*. *Sep Purif Technol* **62**:480–483 (2008).
22 Mandal V, Mohan Y and Hemalatha S, Microwave assisted extraction – an innovative and promising extraction tool for medicinal plant research. *Phcog Rev* **1**:7–18 (2007).
23 Makkar H, *Quantification of Tannins in Tree and Shrub Foliage: A Laboratory Manual*. Kluwer Academic Publishers, Dordrecht (2003).
24 Mercado-Martínez D, Estudio de la recuperación de ácido nordihidroguayarético por cultivos fúngicos de *Larrea tridentata*. Master thesis, Autonomous University of Coahuila, Satillo, Mexico (2008).
25 Szabo MR, Iditoiu C, Chambre D and Lupea AX, Improved DPPH determination for antioxidant activity spectrophotometric assay. *Chem Papers* **61**:214–216 (2007).
26 Li H, Bo C, Zhang Z and Yao S, Focused microwave-assisted solvent extraction and HPLC determination of effective constituents in *Eucommia ulmodies Oliv. (E. ulmodies)*. *Talanta* **63**:659–665 (2004).
27 Sun Y and Wang W, Ultrasonic extraction of ferulic acid from *Ligusticum chuanxiong*. *J Chinese Inst Chem Eng* **39**:653–656 (2008).
28 Zhou H-Y and Liu C-Z, Microwave-assisted extraction of solanesol from tobacco leaves. *J Chromatogr A* **1129**:135–139 (2006).
29 Xiao W, Han L and Shi B, Microwave-assisted extraction of flavonoids from *Radix Astragali*. *Sep Purif Technol* **62**:614–618 (2008).
30 Gao M, Huangb W, RoyChowdhury M and Liu C, Microwave-assisted extraction of scutellarin from *Erigeron breviscapus* Hand-Mazz and its determination by high-performance liquid chromatography. *Anal Chim Acta* **591**:161–166 (2007).

